Hydroxyapatite Coating of Detachable Coils for Endovascular Occlusion of Experimental Aneurysm

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Summary

The purpose of this study was to evaluate the effect of hydroxyapatite (HAp) coating on Guglielmi detachable coils (GDCs) in an experimental aneurysm model. A total of 12 aneurysms were experimentally made in the common carotid arteries of swine using a microsurgical technique. Embolization was done on these aneurysms using standard GDCs and GDCs coated with HAp (GDC-HAp). The animals were then killed 14 days after embolization. The physical properties of coated coils and the development of tissue scarring and coverage the aneurysm's orifice were evaluated macroscopically and microscopically. Macroscopically, a scar formation and coverage at the neck of aneurysms were observed in a group with GDC-HAp, while such findings were not seen in a group with GDC. With light microscope, fibroblasts were seen in the neck of the aneurysms in a group using GDC-HAp, whereas only a fibrin-like net was seen in a group using GDC. In a group with GDC-HAp, inflammatory response was more intense in the dome of the aneurysm with faster re-endothelial coverage of the neck of the aneurysm than the ones in a group with GDC. These results indicated that GDC-HAp might create a clinically beneficial biological surface in an experimental aneurysm model.

Introduction

GDCs are clinically adapted as a useful alternative of the direct surgery of intracerebral aneurysms. However, a neck remnant problem, frequently seen in wide-necked, large, or giant aneurysms, remains to be unsolved [3]. Several studies dealing with long-term angiographic and histopathologic examinations showed that the treated aneurysms with small neck remnants developed coil compaction and recanalization and coverage across the neck of the aneurysm was not recognized [1,6,7].

In an experimental study, coating coils with various proteins and extracellular matrix promote healing of wound in a vessel wall [9,13], though it may induce intra-aneurysmal scar formation and re-endothelialization across the neck of aneurysm.

Hydroxyapatite is a biological carrier with enhancement of thrombus organization, although platinum coils were inert and not cell-adhesive. Tissue engineering is the technology of the remodeling of living organisms in vitro and involves the architecture of artificial cellular scaffolds, which mimics extracellular matrix. Apatite formation on/in hydrogel matrices were developed using a novel alternate soaking process [11]. This study was designed to check the

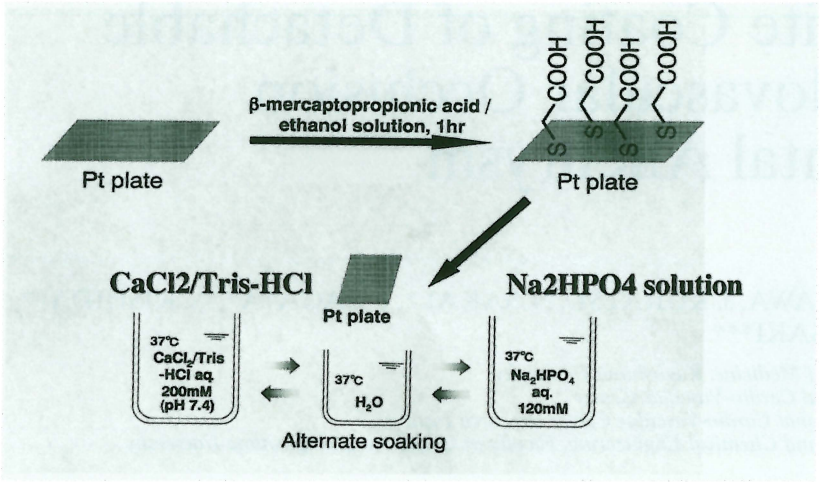

Figure 1 A schema of Hydroxyapatite coating is shown. The platinum plate is coated by alternate soaking.

hypothesis that embolization with coating coils developes the healing process in an experimental intracerebral aneurysm model of swine.

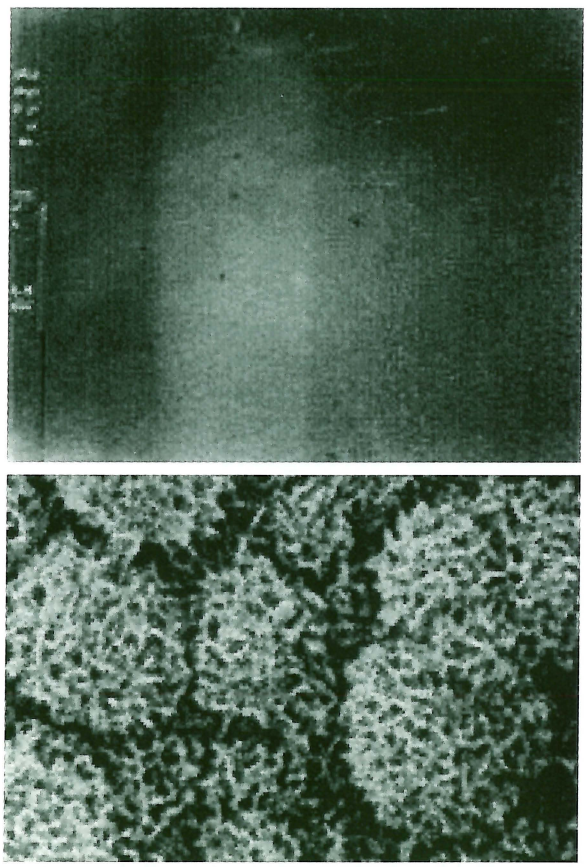

Figure 2 Scanning electron-microscopic appearances of surface of platinum plate (upper) and Hydroxyapatite coated platinum plate (lower) (original magnification, x5000). Note the califlower-like appearance of hydrozyapatite crystals on the surface.

Material and Methods

1) Coil coating

GDC, sizes 18 (platinum coil with 0.015 inches thickness; Boston Scientific Corp., Fremont, CA) was used for embolization. Hydroxyapatite coating method is based on the widely known wet synthesis of hydroxyapatite and is alternative soaking process in CACl2 / Tris-HCl (pH7.4) and Na2HPO4 aqueous solutions. GDC was soaked in 10ml of the CACl2 (Nacalai) (200mM) / Tris (Sigma)-HCl (pH7.4) (Ca solution) at 37°C for 2 h, and was subsequently soaked in Na2HPO4 (Wako) (120mM) aqueous solution (P solution) at 37°C for 2 hours. This procedure was performed five time's over[11] (figure 1).

2) Aneurysm construction and coil embolization

Three to 4-month-old swine with an average body weight of 25.5 kg were premedicated by intramuscular administration of 20 mg/Kg ketamine and 2 mg/Kg xylazine. After endotracheal intubation, general anesthesia was maintained using mechanical ventilation and inhalation of 0.5 to 1.5% halothane.

A total of 12 saccular aneurysms with 5 mm in neck and 6 to 8 mm in length were experimentally made in bilateral common carotid arteries of 7 swines using microsurgical technique. The procedures include 5-mm-long arterectomy, followed by a venoarterial end-to side anastomosis made with 8-0 prolene according to Murayama and Vinuela[14](figure 3). All endovascular embolization were performed immediately after aneurysm was made. A 2.2 Fr

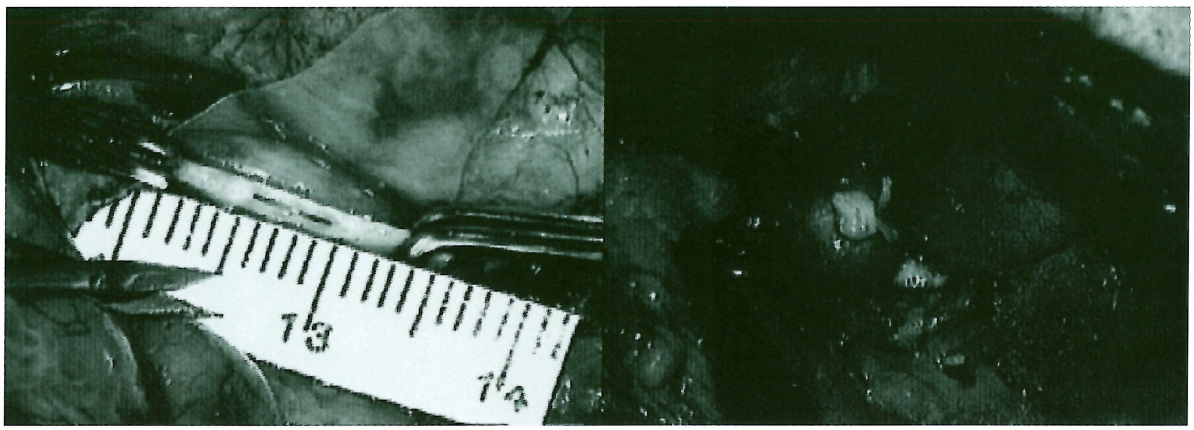

Figure 3 Using a microscope, a 5-mm-long arterectomy was performed and a side wall aneurysm was created by venoarterial end-to side anastomosis.

microcatheter (Fastracker 18, Boston Scientific, Fremont, CA) were positioned with the tip in the aneurysms through the No. 6 French guiding catheter (Fasguide; Boston Scientific, Fremont, CA) via transfemoral approach. GDCs, sizes 18 (6-20 cm length, 3-8 mm helix diameter), were placed in the lumen of the aneurysm until no additional coils could fit into the aneurysm. During procedure, an intravenous bolus of 5000 U heparin was infused systemically to prevent thromboembolic complication on measurement of activating clotting time. After embolization, swine were given 100 mg ticlopidine hydrochloride for two weeks.

3) Final angiographic and histopathologic studies

Final angiography was performed before killing animals to document the radioanatomical results. Then the aneurysm-parent artery complex was removed and parent artery of the specimens were cut from the bottom to the orifice and direct view of aneurysm orifice and coil surface was obtained and photographically documented. Craniotomy was made and rete mirabile was removed after perfusion with heparinized saline. The specimens were fixed with 2% formaldehyde and embedded in methylmethacrylate. Histological sections were cut using diamond band saw. Longitudinal sections of aneurysm were obtained through the center of aneurysm neck and surface stained with hematoxylin and eosin. Histological image of thrombus around the coils in the body and re-endothelialization across the neck was analyzed between aneurysms embolized by standard GDC and those embolized by GDC-HAp.

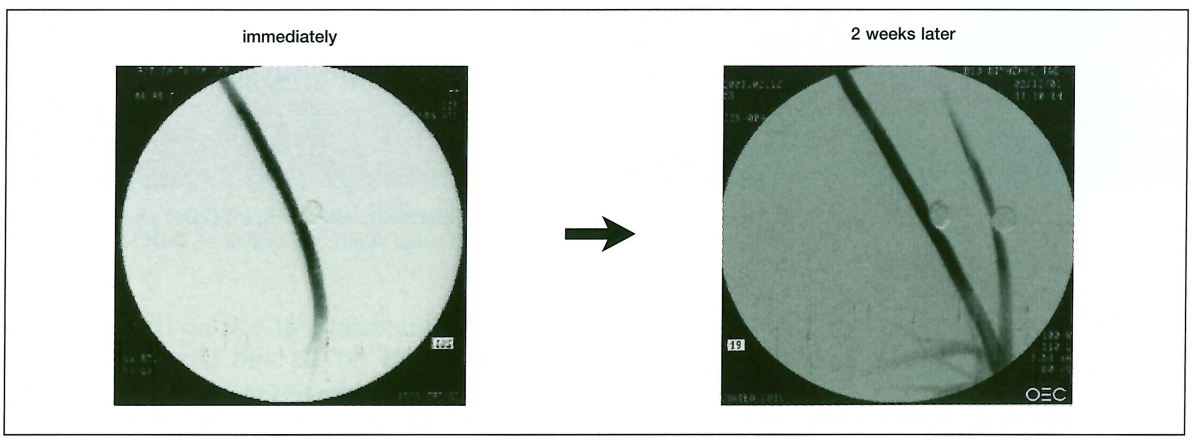

Figure 4 Angiograms of experimental aneurysms created on bilateral common carotid arteries and occluded aneurysms with GDC-HAp. After tight packing of aneurysms, complete occlusion was recognized 2 weeks later.

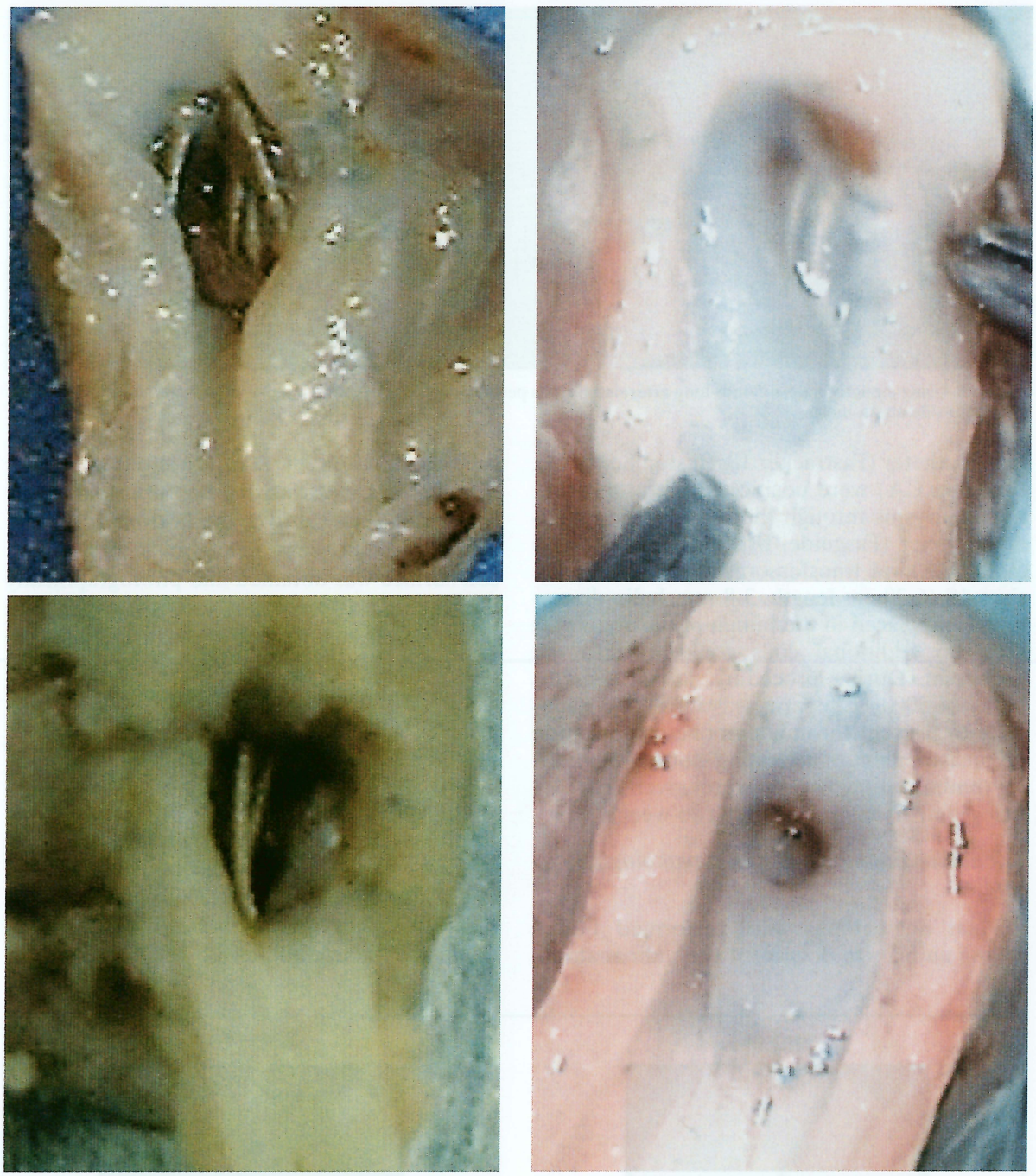

Figure 5 Macroscopic appearances of an aneurysmal orifice 14 days after treatment, with standard GDC and GDC-HAp. Surfaces of standard GDCs were covered with fibrin-like materials. On the other hand, the orifice of GDC-HAp treated aneurysm was covered with thick fibrous tissue.

Results

1) Macroscopic and microscopic findings

In the physical characteristics of GDCs during embolization, such a softness, smoothness, and memory shape, there were no unfavorable change. Coil detachment was no difference between standard GDCs and coated GDCs. In vitro, SEM surface appearance of platinum plate and HAp coated plate demonstrated more extensive surface area in HAp coated plate than in platinum plate (figure 2).

2) Angiographical studies

Embolization was performed for 12 aneurysms. Total length of coils used in each aneurysm were from 28 cm to 58 cm (mean, 42.5 ±5.2 cm), volume ratio of coils with respect to aneurysmal volume were approximately 15% to 25%. Volume ratio of coils; Length of coils× (coil radius) 2×π /sac volume.

Fourteen days after embolization, angiographic follow-up in both 2 of four aneurysms embolized by standard GDCs and 6 of 8 aneurysms used by coated GDCs demonstrated complete occlusion of the aneurysms (figure 4). 2 of the aneurysms showed recanalization. 2 aneurysms had parent artery stenosis and thrombus formation from orifice to parent artery.

Macroscopic examinations of the aneurysms showed significant differences between the standard GDC and the Coated GDCs (figure 5). The surface of the standard GDCs was covered with a thin fibrin-like white material. On the other hand, a denser and thicker reddish fibrous tissue response was observed at the neck of aneurysms embolized with HAp-GDCs. The predominantly fibrous scarring was covered with neoendothelium arising from the edges of the neck of the aneurysm.

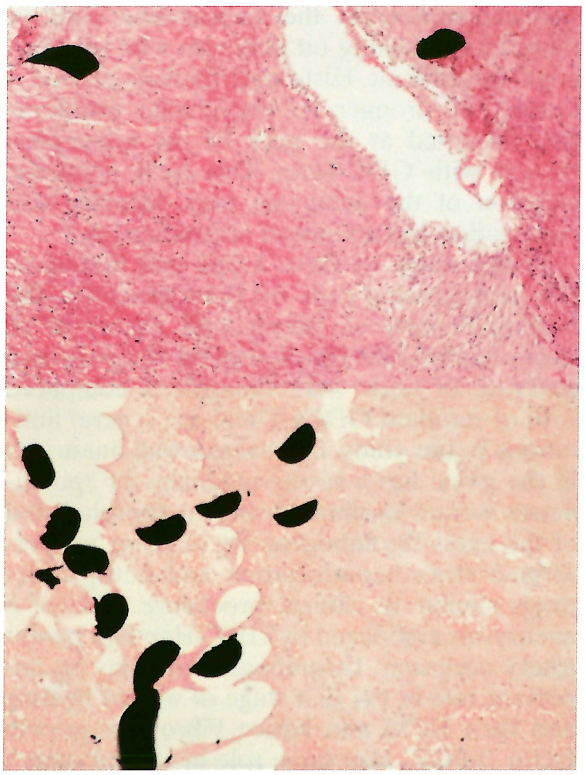

Figure 6 Light microscopic findings in a region of the aneurysm sac. The aneurysm sac treated with GDC-HAp was filled with fibroblasts and monocytes (lower). Fibrin nets were seen with standard GDC (upper), whereas a microscopic mild fibroblast growing was seen in the aneurysmal dome with GDC-HAp.

3) Light and Microscopic Findings

Light microscopic findings on the histologic specimens are detailed in figure 6. Lower-magnification light microscopy showed well-organized fibrous tissue bridging the necks of the aneurysms embolized with HAp-GDCs. A thin fibrin-net around the coils and mild inflammatory cell response were seen in those aneurysms embolized with standard GDCs. Higher-magnification light microscopy showed mild and organized fibrous tissue reaction as well as fibroblasts surrounding HAp-GDCs near the necks of the aneurysms. Interestingly, microscopic neovascularization was seen in the dome of aneurysms.

Discussion

Clinical trial have shown that anatomical outcome of incomplete embolization is a potential risk of aneurysm recanalization. Several studies have stated that important anatomic limitations associated with this endovascular therapeutic procedure [5]. Platinum is inert biologically and does not elicit a significant biological response on its surface. Tamatani et al [12]) demonstrated no endothelial cell proliferation on the platinum coil surface in vitro. Bavinsky, et al [1] suggested that six of 12 complete and permanent aneurysm occlusions on initial angiography showed tiny open spaces between the coils at the neck on gross examination. Biologically active coils may improve the delay in the aneurysm scar formation and might stimulate intra-aneurysmal and neck inflammatory response with concomitant neoendothelial coverage.

Several investigators have used coating by polyester fiber [4], collagen cores [2], incorporating collagen thread [10], ion implantation with fibronectin,laminin [8] to modify coil thrombogenicity and inflammatory cellular response related to their intravascular implantation. Those increased coil thrombogenicity but also changed its physical characteristics. The effect

was imcomplete in the surrounding coils in those units. Coating on platinum with proteins might be difficult. Histologic findings proved a faster and stronger neoendothelial proliferation observed at the neck of the aneurysms treated with GDC-HAp. This early anatomic isolation of the aneurysm may decrease coil compaction and aneurysm recanalization.

In this study, we have found that embolization with coating coils by HAp promotes the healing process of lateral wall aneurysm of swine model. Fibroblasts around the coils and covering of neck were recognized compared with of standard GDCs. There are several limitations in our study. Lateral sidewall aneurysm in the swine has a strong tendency for spontaneous wound healing. Owing to apprehension of the exess thrombogenecity of HAp, we performed strict heparinization and anti-platelet drugs similar to clinical procedure. One of 8 aneurysms embolized by GDC-Hap had parent artery occlusion. Thrombogenecity play an important role in the first stage of wound healing and then ECM and basicc Fibroblast growth factor (bFGF) play same role in second stage [9]. Our study showed the difference of anatomic and histologic changes in aneurysms embolized with standard GDCs versus GDCs-HAp in the early stage. Some studies have examined the effect of various proteins on SMC proliferation, attachment, and the growth of endothelial cells derived from artery wall [12]. Using HAp for biological carrier of proteins, further evaluation that surfice GDC-HAp were coated by proteins such as bFGF is now under investigation.

Conclusion

HAp-coated GDC share similar physical characteristics with standard GDCs. Experimental aneurysms treated with HAp-coated GDC show a faster biological response in the aneurysm body and dome and faster neoendothelial proliferation and migration at the neck of the aneurysm. Such promoted biological response may decrease the chances of coil compaction and recanalization in human cerebral aneurysms treated by GDCs

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